RIBOSOMAL DNA VARIATION WITHIN AND AMONG PLANT POPULATIONS[1]

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ABSTRACT

The DNA sequences that code for the 17S, 5.8S, and 25S subunits of the ribosome have been useful in the study of plant evolutionary biology. The coding sequences are evolutionarily conservative and have provided information on systematic relationships among the species within a genus and have also elucidated higher level relationships. The intergenic spacer region of the sequence is highly variable, and variation occurs within populations and individuals. Analysis of intra- and interpopulation variation in rDNA has documented subdivision within populations of Clematis fremontii *and population and subspecific differentiation in* Phlox divaricata. *These studies indicate that rDNA provides a good genetic marker for the study of microevolutionary processes.*

Many of the current questions in population biology center on the levels of genetic variability within populations and on the factors that influence genetic variation. Levels of genetic variability are central to the study of population biology and evolution because the amount of variability directly influences the evolutionary potential of populations and species. Much attention has been given to the problems of measuring genetic variation for different features, such as morphology, life-history traits, chromosomes, and various types of molecules (e.g., Lewontin, 1974).

In recent years, a predominant technique for examining genetic variation has been allozyme electrophoresis. This technique has greatly expanded our understanding of the genetic processes that occur in plant species, and without it we would have information on the genetic structure of only a handful of noncultivated species. In spite of its usefulness and widespread application, there are some well-known limitations. Most frequently, only genes of a single class, those encoding soluble enzymes, are analyzed, and they often are selected on the basis of the ease of their products extraction and ability to migrate on a starch gel. Only nucleotide differences in genes that lead to changes in product amino acid composition can be detected, and then detection is usually limited to those changes in amino acid composition that result in a net change in charge of the product molecule. These genes may not be representative of the genome in general. For instance, there is evidence that many commonly studied allozymes are more variable than other categories of gene products, and that this variability may stem in part from such processes as post-translational modification (Johnson, 1979). Moreover, changes in allele migration were thought to result from single codon changes; in fact, differences may be the result of several changes in DNA structure (Sachs et al., 1986). Many of these concerns can be avoided if DNA that encodes different types of genes is analyzed.

Plant DNA is relatively simple to extract and purify, and DNA representing different portions of the genome can be studied by hybridization to cloned probes. In addition, current techniques of DNA analysis are many times more sensitive to changes in gene structure than are other macromolecular assays. Variation in fine structure can be detected at several levels: in nucleotide sequence, in sequence length, and in gene copy number. Finally, current DNA technologies are straightforward and require only small amounts of tissue. It is thus feasible to analyze the large numbers of individuals required for populational studies. The use of restriction site and sequence data offers

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the potential to reexamine once-problematic aspects of population biology. Accurate measures of genetic variation in specific portions of the genome, determination of the amount of genetic change associated with speciation, and assessment of the amount of somatic variation and its influence on population variation are examples of areas where DNA data can provide new insights.

Populational analysis offers new insights into molecular biology as well, for example, in the study of concerted evolution. Concerted evolution takes place when the members of a multigene family are more similar to each other than expected had they evolved independently from the time of the initial gene duplication that gave rise to the multigene family (Zimmer et al., 1980; Arnheim, 1983). If concerted evolution were not occurring, each individual would have a large amount of variation among the copies of a multigene family; virtually none of the copies within an individual would be the same. Early DNA hybridization studies and subsequent populational analysis indicated that this is clearly not the case for ribosomal DNA (rDNA). Most of the rDNA copies within an individual are much more similar than would be expected had they evolved independently of one another (a limited amount of variation is seen within individuals, see below, but most rDNA repeats in an individual contain very similar sequences). It is thought that gene conversion, unequal crossingover, or a combination of these are likely responsible for concerted evolution of ribosomal genes. The balance of the processes governing both concerted evolution and the turnover of multiple copy DNA families (Dover, 1982, 1987; Dover & Flavell, 1984) and their interaction with population level phenomena (e.g., gene flow, genetic drift, and organismal selection) are complex and require much additional study. The pattern of variation in specific DNA sequences within and among populations will provide information on the pattern of molecular changes among rDNA repeats.

In the following we will examine variation in DNA sequences within and among populations of plant species. Our purpose is two-fold: to quantify the type and levels of variation at specific DNA sequences and to relate levels of variation to populational features. We will concentrate on one specific DNA sequence, ribosomal DNA, that has received a great deal of attention from molecular biologists and, most recently, population biologists. Before turning our attention to ribosomal DNA variability, we will discuss the structural and functional aspects of rDNA that affect levels of variation.

Structure and Functional Aspects of rDNA

Ribosomal DNA is a mid-repetitive sequence with from 500 to more than 40,000 copies per genome arranged in tandem repeats (Long & Dawid, 1980; Rogers & Bendich, 1987a). Ribosomal gene repeating units are composed of a number of regions that vary in functional constraint and, consequently, in evolutionary rate (for a review see Gerbi, 1986). Figure 1 shows the segments of the rDNA that will be discussed here. Each rDNA repeat contains a transcription unit (a through f), from which the rRNA precursor (pre-rRNA) is transcribed, and a so-called nontranscribed (or intergenic) spacer (g) between the transcription units of adjacent repeats. The pre-rRNA is cleaved after transcription into the mature rRNAs: the 17S (b), 5.8S (d), and 25S (f). The sequences of the rDNA that correspond to the mature rRNAs are the coding regions. The 5′ leader sequence (a) is the external transcribed spacer (ETS). The internal transcribed spacers separate the 17S, 5.8S, and 25S RNA coding sequences (ITS-1 [c] and ITS-2 [e], respectively). The pre-rRNA is transcribed and processed into the various rRNAs. Ribosome subunits are assembled from these gene products along with the 5S rRNA and the ribosomal proteins.

The different evolutionary rates observed for the various regions of rDNA is a likely reflection of the differences in the functional constraints that govern these regions. Portions of the coding regions have a high degree of evolutionary conservation, being invariant in all organisms examined to date. The nontranscribed spacer (NTS), on the other hand, diverges among closely related taxa. Other regions of the repeat show a range of intermediate rates. The rates of divergence determine a specific sequence's utility for studying the variation among populations or higher taxonomic levels. We will review the functions of the various coding and noncoding regions and then discuss the sorts of variation seen for portions of the rDNA repeats in plants.

CODING REGIONS

The coding regions, segments of the rDNA repeat ultimately incorporated into the cytoplasmic ribosomes, are expected to vary the least. Although this is true as a rule, limited variability is possible because of a range of functional constraints. Selection appears to act to conserve functionally important secondary structure (Wheeler & Honeycutt, 1988). Higher amounts of variation at the sequence level are seen among closely related taxa

FIGURE 1. *rDNA repeat unit organization. The line corresponds to the nontranscribed spacer region (g), while boxes (hatched or filled) represent the transcribed portion of the gene. Filled boxes represent the coding regions for the 17S (b), 5.8S (d), and 25S (f) rRNAs. Hatched boxes symbolize the transcribed spacers, the external (ETS, a) and internal (ITS-1, c, and ITS-2, e). The repeating structure of these genes is represented by the adjacent ETS (a') and 17S region.*

for the portions of the rRNAs constituting the helical 'stem' portions of the molecule. Changes may occur by compensating substitutions, in which base-paired nucleotides in opposite strands of the helix change 'in response' to one another. A degree of mismatch is apparently tolerated. Presumably occasional mismatches may slow down the rate of formation of stem and loop structures without preventing the formation of the helix. In addition to guanine-cytosine and adenine-uracil pairing, pairing between guanine and uracil is possible and does not inhibit helix formation. Furthermore, even short stretches of one or a few nucleotides of mismatch do not prevent formation of helical structure as long as they are flanked by regions of base complementarity. The most conserved sequences are in single-stranded regions (Wheeler & Honeycutt, 1988). These sequences either act enzymatically or bind to proteins (either ribosomal proteins or protein translational cofactors) or other RNAs (tRNAs and mRNAs).

Some stretches of rRNA sequence do not vary in any organism for which the sequence is known. At the other extreme, portions of the large ribosomal RNA are either variable within an individual (Gonzalez et al., 1985) or present in some species of a genus but are lacking in others (Chan et al., 1983; Hadjiolov et al., 1984). Although the evolutionary dynamics of these changes is not understood, obviously there is polymorphism within populations for rRNA coding region variants. Most studies of rDNA variation analyze restriction endonuclease sites, and little variation has been reported for sites within coding regions. Sequence analysis would be much more sensitive for detecting variation, but with the exception of Gonzalez et al. (1985), most rDNA sequences are known for only a single gene copy of each species. The expected level of variation is too low to justify such a currently expensive and labor-intensive survey.

TRANSCRIBED SPACER REGIONS

The transcribed spacer regions are the portions of the rRNA transcription unit that are not seen as mature rRNAs. They show intermediate levels of variability in interspecific studies (Appels & Dvorak, 1982b; Sytsma & Schaal, 1985; Hillis & Davis, 1986; Davis, 1986), an observation consistent with the intermediate degree of functional constraint on these sequences. While some portions of the transcribed spacers may act merely as spacer DNA with the length of the sequence being more important than its information content, analysis of ITS sequences shows substantial conservation among moderately closely related species. Presumably this reflects the presence of processing signals, for which a degree of conservation is expected. Sequence conservation is also seen for portions of the ETS, again presumably due to processing signals in this region. In addition, it has been postulated that the intermediate level of conservation in transcribed spacer regions may reflect RNA-mediated gene conversion; Appels & Dvorak (1982b) suggested that the conservation seen for rDNA may be due in part to 'correction' from the rRNA transcript of differences among ribosomal gene copies within the same nucleolus.

NONTRANSCRIBED SPACERS

This region between adjacent transcription units is in fact transcribed to a degree, so it is becoming apparent that this term is a misnomer. Transcription proceeds from the 5′ transcription unit, through the spacer, to the initiation site of the adjacent repeat; these transcripts are rapidly processed to the rRNA precursor and the ephemeral nature of NTS transcripts led to this region being so-named. The NTS is the most rapidly evolving portion of the rDNA. Since it shows the greatest amount of variation within and among plant populations, it is the region most useful as a genetic marker for analyzing microevolutionary processes. The dynamics of molecular evolution in the nontranscribed spacers in plants have begun to be elucidated recently. From sequencing studies (Appels & Dvorak, 1982a; Yakura et al., 1984; Lassner & Dvorak, 1986; McMullen et al., 1986; Toloczyki & Feix, 1986) and other fine-scale genetic analyses (Appels & Dvorak, 1982b; Rogers et al., 1986), it appears that the NTS consists of at least three regions that may differ in function and may evolve at different rates.

A series of subrepeating elements in the nontranscribed spacers is seen in all higher eukaryotes for which the sequence is known. In addition, the presence of subrepeats in a number of plant species is inferred from length variability in the NTS (e.g., Cluster et al., 1984, reviewed by Rogers & Bendich, 1987a). A degree of sequence similarity has been demonstrated between NTS subrepeats of wheat (Appels & Dvorak, 1982a) and maize (McMullen et al., 1986; Toloczyki & Feix, 1986). This presumed conservation has been interpreted as evidence that the subrepeats have a function. It has been demonstrated that one class of subrepeating elements acts as enhancers of transcription in *Xenopus* (Reeder et al., 1983; Reeder, 1984), and evidence suggests that some types of subrepeats function similarly in plants (Flavell & O'Dell, 1979; Martini et al., 1982; Flavell, 1986).

No function has been demonstrated for the region of the NTS 5′ to the presumed enhancer subrepeats. This sequence is 144 base pairs in maize (Toloczyki & Feix, 1986) and at least 241 bp in wheat (Lassner & Dvorak, 1986) but may be considerably longer in other plant taxa. The region 3′ to the enhancer subrepeats is 135 to 240 bp in maize (McMullen et al., 1986; Toloczyki & Feix, 1986); in wheat it is considerably less than 960 bp (Lassner & Dvorak, 1986). This region is assumed to contain the promoter for transcription of the pre-rRNA. Although the NTS is presumed to code for no gene products, there is good evidence for functional constraints, and the NTS is therefore potentially subject to selectional forces. It is not clear how strongly these constraints govern the evolution of the NTS region of the rDNA multigene family, but they clearly differ from those governing evolution of the coding regions (see Jorgensen, this volume, for further discussion).

Variation among Individuals of a Population

Because of these differences in the levels and kinds of functional constraints, variation of rDNA is very different for the transcription unit versus the so-called nontranscribed spacer. In general, within a species there appears to be only little variation in the coding regions. Such variation appears to be predominantly developmental variation due to methylation (see Jorgensen & Cluster, this volume). We will concern ourselves here with variation in the nontranscribed spacer region. When the individual plants within a population show some type of rDNA variation, it is within this region. Length variation is most common and is due to a series of repetitive elements in the nontranscribed spacer region. In *Triticum* spp. and *Vicia faba*, length heterogeneity is due to copy number differences of a series of 135-bp or 325-bp elements, respectively (Appels & Dvorak, 1982a; Yakura et al., 1984). A variable number of copies of the same or highly similar DNA sequence gives rise to the different length classes.

Because rDNA is a repetitive DNA sequence within the genome, individuals can contain several different length variants. A single *V. faba* plant can have up to 20 different length variants of rDNA (Rogers et al., 1986). Native populations of *Lupinus texensis* contain plants with up to 11 length variants, although most commonly there are three or four variants per plant (D. Baum, pers. comm.). In *Phlox divaricata*, the mean number of repeats per plant is 1.98 (Schaal et al., 1987). *Clematis fremontii* has an average of 2.65 variants per individual (Learn & Schaal, 1987), whereas *Hordeum spontaneum* contains on average 2.28 variants per plant (Saghai-Maroof et al., 1984).

Such length variation is not ubiquitous. *Solidago altissima* is highly variable in the nontranscribed spacer region but this variability is limited almost exclusively to restriction site variation (Schaal et al., in prep.). Length variation is restricted to a 300-bp insertion present in low frequency within some populations. Table 1 shows the variation in restriction sites of *S. altissima*. Variation of rDNA occurs often within individual plants. Plants are most commonly polymorphic for rDNA variants that have different restriction sites. Another feature of *S. altissima* rDNA is the genetic differentiation among portions of a clone. Several plants showed variation in rDNA types within a clone for variants based on different *Eco*RI or *Eco*RV restriction sites. Such within-clone differences may occur via somatic mutation or rapid increase of a rare variant. The occurrence of variation within plants adds a level of analysis not previously possible in populational studies.

Other species show no rDNA variability. *Rudbeckia missouriensis*, an endemic of isolated rocky habitats in Missouri and Arkansas, shows no length heterogeneity nor does it show restriction site variation in a survey of six populations of glade habitats (L. King, pers. comm.). *Gaura demareei*, a hybrid-derivative species with a narrow range, contains two length variants of 10.5 and 11.3 kb. Each plant examined in a survey throughout its range was identical for the two length variants (Schaal & Raven, in prep.). Moreover, there was no restriction site variation. Similarly, species of the

Lisianthius skinneri complex show little restriction site variation or length heterogeneity (Sytsma & Schaal, 1985).

At this time no clear correlations emerge between levels of rDNA length variability and characteristics of the population biology of various species. In the three cases where no variation is observed, the species are narrowly endemic. The *R. missouriensis* populations are isolated, although population size can be in the thousands. *Gaura demareei* has a highly restricted species range, occurring predominantly within a single Arkansas county in populations often fewer than 50 individuals. Likewise, *Lisianthius* species have a very narrow range and often consist of few populations with low plant numbers. On theoretical grounds one expects that narrowly endemic species would have little variation, due to genetic bottlenecks. Variation would be expected to be lost due to sampling, in these cases either by small population size, by founder events due to repeated colonizations, or by a recent species origin after hybridization. The generation of length heterogeneity may not occur very rapidly in these species since none of them have accumulated variation; even *R. missouriensis*, where number of individuals per population is high, remains depauperate for rDNA length variation.

In marked contrast to the results obtained for *R. missouriensis* are the levels of variation detected in *Clematis fremontii*. Populations of *Clematis fremontii*, like the *Rudbeckia*, occur on islandlike glades in Missouri. These populations are predominantly limited to two counties and are often much smaller in population number than *R. missouriensis*. On theoretical grounds one would expect *C. fremontii* to show less variation than *R. missouriensis*, but this is not the case. The *Clematis* plants contain up to four length variants, and the length variation shows spatial differentiation (see below). Thus, there is no consistent pattern here between ribosomal DNA variation and population size or species range (but see Flavell et al., 1986). Since the two species with the greatest observed length heterogeneity are both legumes (*Vicia faba* and *Lupinus texensis*, see above), one might suspect that something in the ancestry or in the biology of legume species leads to such high numbers of rDNA length variants, but length heterogeneity is not great in some other legume species (soybean and its relatives, Doyle & Beachy, 1985; other *Vicia* species, Rogers & Bendich, 1987b). Clearly, much more research is necessary before associations between levels of rDNA length variation and populational characteristics, such as gene flow, population size, breeding system, or founder events can be established.

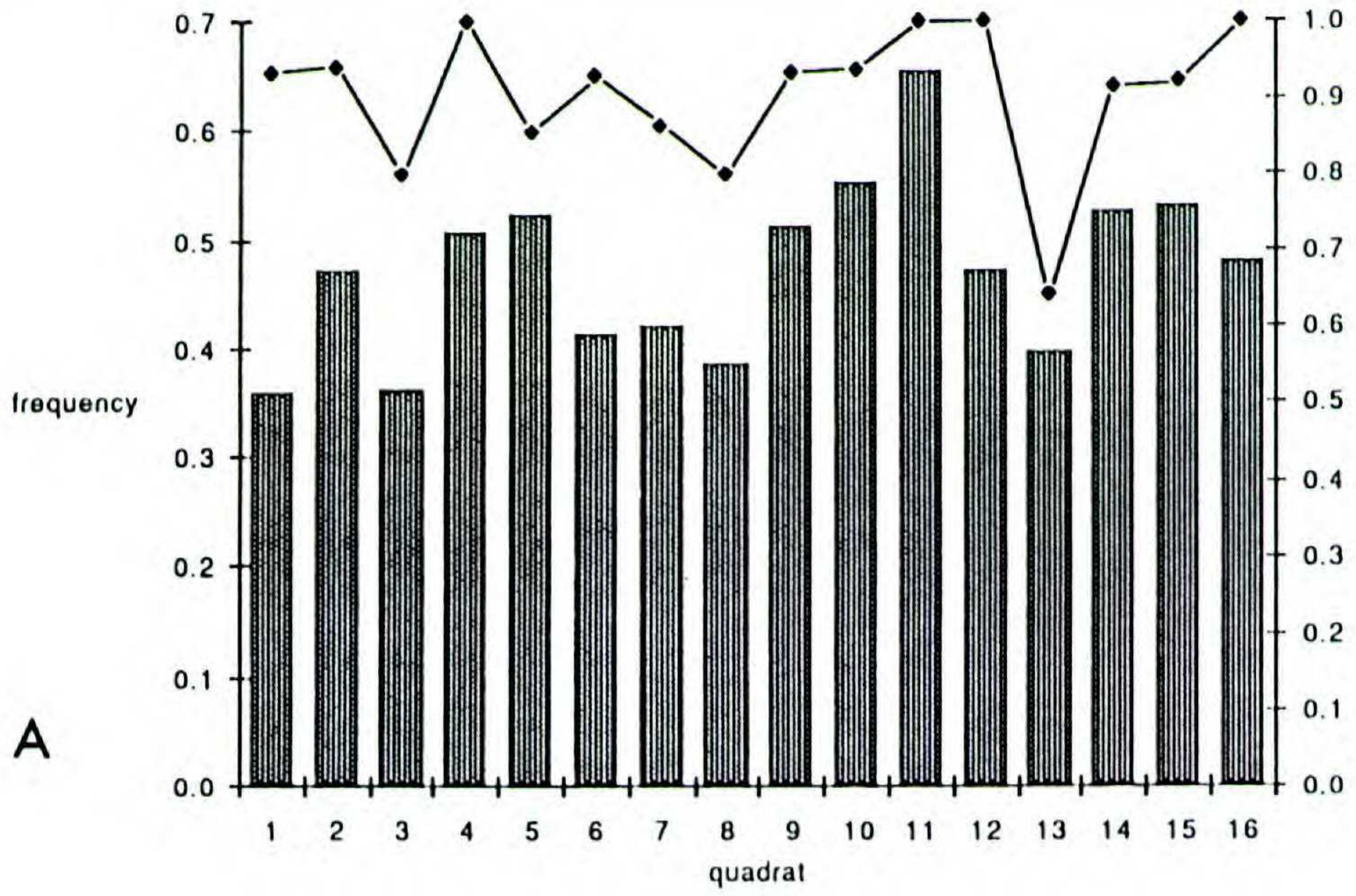
TABLE 1. *Variation of rDNA in* Solidago altissima.

Site	Within Individuals[1]	Within Clones[2]	Between Individuals	Between Populations
*Sst*I	+	−	−	−
*Bgl*I	+	−	−	−
*Bgl*I	+	−	−	+
*Xmn*I	+	−	+	−
*Hinc*II	+	−	−	−
*Xho*I	+	−	−	−
*Bam*HI	+	−	−	−
*Eco*RI	+	+	−	−
*Eco*RV	+	+	+	−
*Xmn*I	+	−	+	+
*Hind*III	+	−	−	+
Insert	+	−	+	+

[1] Genome contains more than one rDNA variant.
[2] Differences in rDNA type occur among the parts of a clone.

A further aspect of rDNA variation within populations is apportionment and distribution in space. One feature that distinguishes many plant populations from most animal populations is the frequent occurrence of genetic population substructure in the former. Plant populations often show significant local genetic differentiation, many times on a microgeographic scale. Such local differentiation can result from selection on a very local scale. Local differentiation may also occur via genetic drift. Such drift can be the consequence merely of nonrandom mating due to restricted pollinator behavior or to limited seed dispersal—spatially restricted gene flow causes the population to become effectively subdivided. Random genetic drift occurs among the subdivisions, thus leading to significant local genetic heterogeneity (Turner et al., 1982). Local differentiation within plant populations has been documented for genes that cause heavy metal tolerance (Jain & Bradshaw, 1966), that result in different flowering times (McNeilly & Antonovics, 1968), that cause morphological differences (Linhart, 1974), and that encode different allozymes (Schaal, 1975).

Our study of *Clematis fremontii* has documented nonrandom geographical distribution of ribosomal DNA variants within populations. A single population of *Clematis fremontii* has been analyzed for spatial variation in the frequency of rDNA-length variants. Many of the length variants that


0.7
0.6
0.5
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0.0
frequency
1.0
0.9
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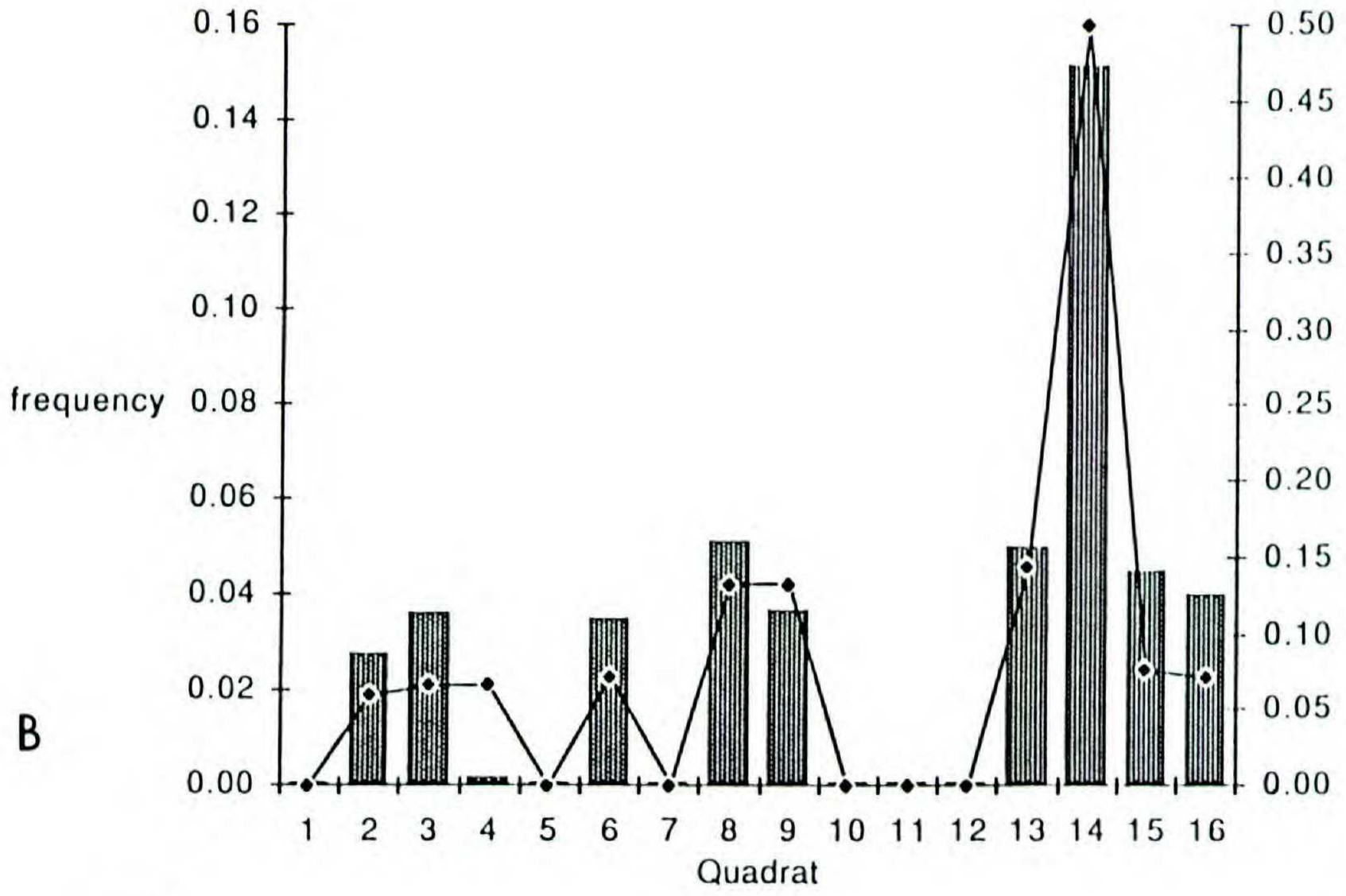
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
quadrat

A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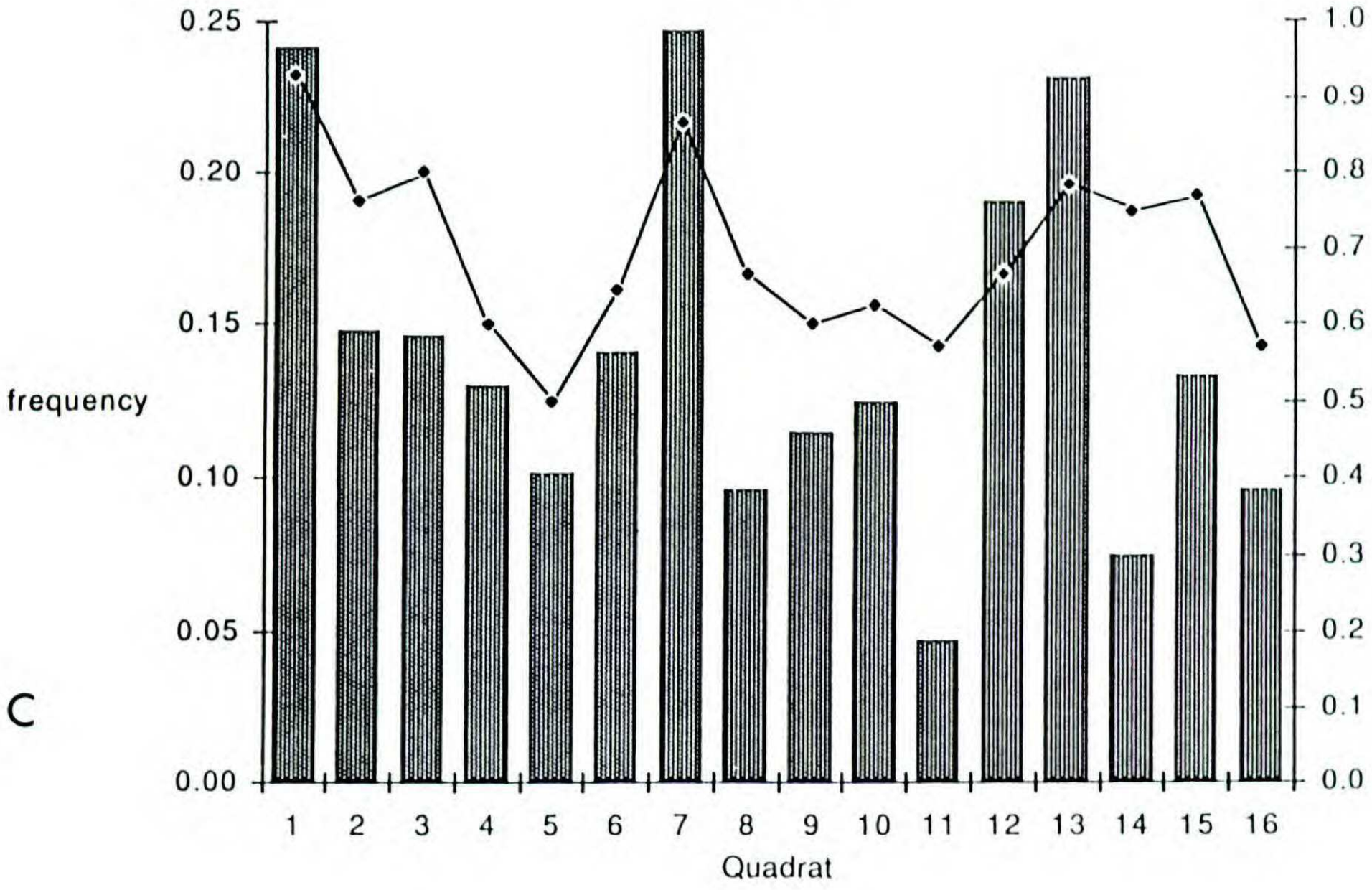
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Quadrat

B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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Quadrat

C

occurred in high frequency showed no significant spatial differentiation (Fig. 2A). However, two of the variants showed statistically significant microgeographic differentiation; the variants do not tend to be distributed randomly in space within the population, but rather are confined to specific areas within it (Figs. 2B, C). Such local differentiation of rDNA variants is consistent with population subdivision due to restricted gene flow, or perhaps it may be a consequence of a recent origin or dispersal of an rDNA variant into a population.

Variation among Populations

Few studies have examined the pattern and apportionment of rDNA variants among the populations of a species. Most of the work to date has centered on cultivated species and is reviewed in Appels & Honeycutt (1986). Here we look at levels of variation in natural, noncultivated plant species. Levels of differentiation for rDNA variants vary among populations of a species. Some plant species show no significant heterogeneity within or among populations. Those species having low levels of rDNA variation within populations show little or no differentiation among populations. No significant genetic differences in rDNA types were detected among populations of *Gaura demareei*, *Rudbeckia missouriensis*, or members of the *Lisianthius skinneri* complex. Judging from their ranges and/or other determinations of genetic variability, it is likely that these species have undergone genetic bottlenecks and variation has been lost within and between populations. In the few highly variable examined species, significant genetic heterogeneity is detected among populations.

The best-studied example to date is the widespread woodland perennial *Phlox divaricata* (Schaal et al., 1987), in which there is clear differentiation of rDNA variants. Populations often contain unique rDNA variants and may be distinguished by the number of variants (2–6) they contain (Schaal et al., 1987). There is clear differentiation between population systems. *Phlox divaricata* subsp. *laphamii* shows less rDNA diversity than subsp. *divaricata*. The subspecies differ in the numbers and types of variants they contain, and in the overall genetic diversity (Table 2).

The variation in *Phlox divaricata* provides corroborative information on the origin of the subspecies. Based on morphological criteria, subsp. *laphamii* is considered derived from subsp. *divaricata*. This hypothesis appears to be supported by the apportionment of rDNA variation; rDNA variability in subsp. *laphamii* is a subset of the variability seen in the other, more widespread subspecies.

Variation among populations also has been analyzed in the old-field perennial *Solidago altissima* (Schaal et al., in prep.). Differentiation in this species occurs for a 200-bp sequence which is fixed in one population and is present in low frequencies in other populations. Populations are also differentiated for restriction sites. As with intrapopulation variation, too few species have been analyzed to draw conclusions about levels of rDNA variation and such populational characteristics as size, gene flow, or bottlenecks. Clearly, populations are differentiated for levels and kinds of rDNA variation. Whether the differentiation is related to selection, genetic drift, gene flow, or any other population-level genetic process remains to be determined. Although the mechanisms responsible for generating rDNA length variation obviously require further study, such variants can and have been used to reconstruct aspects of the evolutionary history of plants (see also Sytsma & Schaal, 1985; Doyle et al., 1984, 1985).

Conclusions and Prospects

The use of DNA sequences in studies of population biology is in its infancy and holds a great deal of potential for understanding processes and answering persistently elusive questions. The ability to assess variation in a wide diversity of DNA sequences is a major technical advance. Virtually any segment of DNA can be studied, whether it is a coding or noncoding sequence, or is single copy,

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FIGURE 2. *Frequencies of some rDNA repeat length variants in* Clematis fremontii. *Individuals were sampled from a 160-m transect at Victoria Glade, Jefferson County, Missouri (see Learn & Schaal (1987) for details). Variants A (Fig. 2B) and E (Fig. 2C) show statistically significant local differentiation along the transect.—A. Variant C, 11.3 kilobase pairs (kb). Numbers along the abscissa refer to quadrat position along the transect. The left ordinate and the histogram bars are mean frequency of variant within individuals in a quadrat. The right ordinate and the line are proportion of the individuals within a quadrat bearing a variant C. Variant C is the most common variant in the population and does not show significant local differentiation.—B. Variant A (10.2 kb). Axes as in Figure 2A.—C. Variant E (11.9 kb). Axes as in Figure 2A.*

TABLE 2. *Population variation in rDNA repeat-type frequency of* Phlox divaricata.

Subspecies and Population	Repeat-Type Frequency							
	V-1	V-2	V-3	V-4	V-5	V-6	V-7	V-8
Subspecies *laphamii*								
CC			0.19			0.50	0.31	
T			0.09	0.27		0.55		
WH				0.18		0.55		
F				0.42	0.25			0.08
Results for subspecies overall			0.08	0.38	0.04	0.24	0.10	0.02
Subspecies *divaricata*								
TC				0.29	0.14	0.21		
PH		0.50		0.20	0.30			
B	0.19			0.19	0.10		0.19	
M	0.21	0.08		0.13	0.12		0.17	
Results for subspecies overall	0.11	0.13		0.16	0.19	0.06	0.10	
Results for all populations	0.070	0.078	0.031	0.239	0.141	0.132	0.10	0.003

mid-repetitive, or highly repetitive DNA. In fact, one strategy of population analysis is to clone random portions of the genome, and study variation of restriction sites in these random sequences (e.g., Hofker et al., 1986).

From the studies discussed above, some DNA sequences, specifically segments of rDNA, vary at the appropriate levels for studies of population processes. This is in contrast to other sequences, such as chloroplast DNA, where variation is usually seen at the interspecific or intergeneric level, and is most informative for phylogenetic studies. There are clear differences among individuals and populations in rDNA. An added dimension to studying variation occurs with the use of mid-repetitive sequences, since single individuals contain many copies of a sequence and thus can themselves be polymorphic. Studies of ribosomal DNA provide an additional level of analysis, that of the individual; the apportionment of variation can be examined at the within-plant as well as the between-plant levels.

Another potentially important aspect of rDNA studies is the ability to detect somatic mutations. There currently is much speculation in the literature on the role of somatic mutation in plant population biology (Whitam & Slobodchikoff, 1981; Gill & Halverson, 1984; Walbot, 1985; Walbot & Cullis, 1985). Several workers suggest that somatic mutation and subsequent variation leads to differences in ecological parameters, such as susceptibility to insect predation. It is argued that somatic variation in DNA sequences may have an adaptive function. These ideas are contested, in part because the frequency of somatic variation is not known. With current methods of analyzing DNA sequences, it is possible to document unequivocally the occurrence and frequency of somatic variation.

A final new area where DNA analysis is potentially important for population biology is rapid genomic change. Many organisms alter their DNA in response to stress, as in the case of gene amplification in response to toxic agents (Schimke, 1983). McClintock (1984) suggested that genome change is a way in which plants routinely deal with stress. Walbot & Cullis (1985) suggested that flexibility is an important feature of the plant genome. Genome flexibility has been demonstrated in flax, where heritable variation in rDNA cistron number is induced by environmental changes (Cullis, 1986). Such heritable changes in genome size have profound implications for population biology. Alteration of genomes in response to environmental variation may contribute to the genetic adaptation of a plant species. Such a process can alter the genetic characteristics of populations and, on a practical level, can confound such experiments as reciprocal transplants. There is much future work on the interface between plant molecular biology, population genetics, and ecology; blending among these disciplines promises to add greatly to our understanding of plant evolution.

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TABLE 2. *Continued.*

Repeat-Type Frequency				Mean Number of Repeats/ Plant
V-9	V-10	V-11	V-12	
				2.0
0.09				1.2
0.18			0.09	1.2
0.25				1.5
0.12			0.02	1.5
	0.15		0.21	2.3
				2.0
		0.04	0.30	2.6
		0.12	0.17	3.0
	0.03	0.05	0.17	2.5
0.051	0.016	0.031	0.108	1.98

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